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BIOAVAILABILITY AND ASSIMILATION OF SEDIMENT-ASSOCIATED BENZO[a]PYRENE BY *ILYODRILUS TEMPLETONI* (OLIGOCHAETA)

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Abstract—Benzo[a]pyrene (BaP)-amended sediment was desorbed by a sequential batch method using an isopropanol solution wash. The observed isotherm showed no evidence of desorption resistance, as indicated by increased partitioning to the solid phase at low concentrations. This was consistent with the prediction of minimal desorption resistance for highly hydrophobic compounds using a biphasic model. Bioavailability of BaP in desorbed sediments was assessed by toxicokinetic measures of uptake, bioaccumulation, and elimination in the deposit-feeding, freshwater tubificid oligochaete *Ilyodrilus templetoni*. Worms were exposed to sediments with BaP concentrations of approximately 26 and 11 μg/g dry weight sediment after desorption for one and three batches, respectively. The *I. templetoni* tissue concentration attained an apparent steady state after approximately one month and resulted in a biota–sediment accumulation factor of approximately 1.3 for both sediments. This is consistent with the paradigm that pore-water concentration predicts the uptake of organic contaminants into lipids despite the literature data showing that the major uptake route for BaP is likely from the ingestion of sediment particles. *Ilyodrilus templetoni* exhibited a high assimilation efficiency (80%) during a single-gut passage, a low elimination rate (k_e = 0.0032 h^{-1}), and negligible biotransformation of sediment-associated BaP.

Keywords—Benzo[a]pyrene   Desorption   Bioavailability   Assimilation   Elimination

INTRODUCTION

Benzo[a]pyrene (BaP) is one of a group of organic compounds called polycyclic aromatic hydrocarbons (PAHs) and is considered to be carcinogenic and toxic to many animals, including mammals. Benzo[a]pyrene is released into the environment as a by-product of the combustion of fossil fuels and other materials (wood, oil). Primary anthropogenic sources of BaP include exhaust from motor vehicles and other gasoline and diesel engines; coal-, oil-, and wood-burning stoves and furnaces; products from petroleum refineries; and cigarette and barbecue smoke. Benzo[a]pyrene is a hydrophobic compound with a moderately large octanol–water partition coefficient (log K_{ow} = 6.04) [1]. On entering aquatic systems, BaP rapidly deposits into the sediment and physically adsorbs on the solid surface or partitions into the organic carbon fraction of the sediment. Many studies have demonstrated that the effect of the soil or sediment-associated contaminants on the receptor is not controlled by the total concentration of the contaminant but, instead, by the fraction that is biologically available [2], which is usually defined as the bioavailability of the contaminant. The bioavailability of sediment-associated BaP determines the potential hazard of this contaminant in the environment.

Bioaccumulation tests are usually used to determine the bioavailability of sediment-associated contaminants and the trophic transfer potential of contaminants in the aquatic environment [3]. It is generally assumed that only desorbed contaminants are biologically available to an organism [4]. That is to say, a contaminant first must be desorbed from sediment particles into pore water or an animal's digestive fluids before it can be taken up by the organism. Therefore, sorption and desorption of the contaminant from sediment particles are very important factors that may determine the accumulation of a

contaminant by a specific organism. Many laboratory and field studies have demonstrated that some fraction of a contaminant desorbs quickly and reversibly, whereas desorption from a second fraction is limited in rate or extent, a phenomenon that is usually termed desorption resistance [5–8]. Recent work suggested that desorption resistance may result from partitioning to soot or soot-like materials that exhibit a very large partition coefficient relative to natural organic matter [9–11]. The equilibrium pore-water concentration of the desorption-resistant compartment is much less than that expected by reversible desorption, which may, therefore, influence the uptake by sediment-dwelling organisms.

Bioavailability of desorption-resistant phenanthrene to the tubificid oligochaete *Ilyodrilus templetoni* was studied, and reduced availability was observed for desorption-resistant phenanthrene [12]. The purpose of the present study was to quantify desorption resistance and bioavailability of the highly hydrophobic BaP. The model presented by Kan et al. [8] suggests that the equilibrium effect of desorption resistance is minimal for highly hydrophobic compounds and that the sediment–water partition coefficient to the desorption-resistant fraction is independent of concentration. The limited influence of desorption resistance for highly hydrophobic compounds is unexpected, in that partitioning to condensed-phase organic carbon would be expected to be greater for highly hydrophobic compounds [11]. Limited observation of desorption resistance for highly hydrophobic compounds may be the result of a balance between the equilibrium partitioning and the slow rate of approach to that equilibrium expected for such compounds—in other words, an artifact of short-term (months or less) laboratory experiments or field exposures.

Many other factors besides equilibrium extent of desorption may influence bioavailability. The rate of desorption, the route of uptake (water, sediment, or food) and the uptake efficiency from each route [2], the ability of the organism to metabolize

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the contaminant [4], and the relative contribution from each route [13,14] may influence the rate of uptake and/or steady-state accumulation of the contaminant. Assimilation efficiency (AE), which is defined as the fraction of the adsorbed products that is incorporated into the body tissue [15], is an important parameter in understanding and modeling contaminant accumulation and trophic transfer in the aquatic environment, especially when sediment ingestion is the major uptake route. Assimilation efficiency was also used in quantifying the bioavailability of sediment-associated phenanthrene and B*a*P by Penry and Weston [16]. Besides AE, elimination of the contaminants by active metabolic process and passive diffusion loss may also influence net accumulation and, thus, influence the observed bioavailability of the contaminants.

In the present study, a series of toxicokinetic experiments was conducted on B*a*P-inoculated and isopropanol-desorbed sediments. Experiments were designed to measure the potential for desorption resistance in B*a*P as well as the uptake kinetics, AE, and elimination of B*a*P in a deposit-feeding, freshwater oligochaete. The goals of the present study were to investigate bioavailability of sediment-associated B*a*P to a bulk deposit-feeding organism and to study the factors that may influence the bioavailability of sediment-associated B*a*P.

MATERIALS AND METHODS

Sediment preparation and test organisms

Sediment from a local source, Bayou Manchac (LA, USA), was employed in the present study. The sediment and the test organism, *I. templetoni* (Southern), used were the same as those used by Lu et al. [12], who examined similar questions with phenanthrene. Sediment inoculation and preparation followed the procedures developed by Lu et al. [12], consisting of a sequential batch desorption method and isopropanol washing. Desorption with this isopropanol solution allows the rapid removal of reversibly sorbed compounds, and this procedure is a very effective method to remove readily reversible contaminant. The final contaminant concentration and the proportion of reversible to desorption-resistant contaminants were controlled by the number of batches of desorption and the time period of each desorption. The total B*a*P concentration in the sediment was primarily unlabeled B*a*P (Sigma, St. Louis, MO, USA), supplemented with radiolabeled [³H]B*a*P (American Radiolabeled Chemicals, St Louis, MO, USA) to increase the sensitivity of analysis. The [³H]B*a*P was dissolved in benzene solution (1 mC$_i$/ml) with specific activity of 50 C$_i$/mmol and purity of 99%. Sediment was inoculated at approximately 60μg B*a*P/g dry sediment and tumbled on a roller mill for three weeks to ensure homogeneous partitioning of B*a*P in the sediment matrix. Aging was not an issue in the present study, because the inoculated sediment would be desorbed sequentially by washing with an isopropanol and electrolyte solution (0.01 M NaCl and 0.01 M CaCl$_2$·2H$_2$O in tap water, 1:1 [v/v]) to get sediment with different B*a*P concentrations and different fractions of desorption resistance. Three batches of the desorbed sediments were prepared (S$_1$ through S$_3$), and sediment–water partition coefficients were measured for all the three desorbed sediments and the originally inoculated sediment both to obtain the desorption isotherm of B*a*P and to characterize the desorption resistance of the sediment-associated B*a*P. Only the originally inoculated sediment exhibited a significantly different sediment–water partition coefficient, which was approximately 40% lower than those of the three

desorbed sediments, showing a small effect of desorption resistance.

Before the uptake experiments were initiated, a preliminary test was conducted to determine *I. templetoni* survivorship and activity in S$_0$ through S$_3$ sediments. If the worms were active and no sediment avoidance (i.e., worms avoid burrowing) as well as no mortality of worms were detected after one week of exposure, the sediment was considered to be safe for subsequent experiments. After several hours of exposure to the originally inoculated sediment with a B*a*P concentration of 60 μg/g, all worms died, indicating a high toxicity of B*a*P at this concentration. No mortality or avoidance of sediment was observed in the three desorbed sediments. Sediments that have been desorbed once and three times (S$_1$ and S$_3$, respectively) were used in the bioaccumulation experiment. The concentrations of these two sediments were 25.85 ± 1.46 (*n* = 4) (± SD) and 10.90 ± 0.41 (*n* = 4) μg B*a*P/g dry sediment, respectively. *Ilyodrilus templetoni* was acquired from the Waterways Experiment Station (U.S. Corps of Engineers, Vicksburg, MS, USA) and then cultured in our laboratory. The worms used in the present study had an average wet weight of 3.59 ±0.59 mg (*n* = 25), a dry to wet ratio of 0.20 ± 0.01 (*n* = 25), and an average lipid content of 8.57% ± 2.07% (*n* = 7) at the beginning of the experiments.

Measurement of organic carbon content

Organic carbon content (f_{oc}) of the sediments was analyzed on a Perkin-Elmer 2400 series II CHN elemental analyzer (Perkin-Elmer, Norwalk, CT, USA) with two replicates for each sediment treatment and 5 to 15 mg of sediment employed for each replicate. Samples were not acidified because of the low inorganic carbon content in the Bayou Manchac sediment.

Measurement of sediment–water partition coefficient and pore-water concentration

Sediment–water partition coefficients of the sediments were measured according to the method of Kan et al. [8] and standard procedures of the American Society for Testing and Materials [17]. An equivalent of 0.5 g of wet sediment (water content, ~40%) was weighed per sample and placed into 25-ml glass scintillation vials. Vials were then filled with electrolyte solution (0.01 M NaCl, 0.01 M CaCl$_2$·2H$_2$O, and 0.01 M NaN$_3$ in deionized water) with minimal headspace and tumbled for 10 d to allow achievement of equilibrium. At the end of 10 d, the sediment slurry was centrifuged for 2 min in 1.5 ml-microcentrifuge tubes at 10,000 *g*, and B*a*P concentration in the water was measured by liquid scintillation counting. The sediment concentration after desorption was calculated by mass balance assuming that no loss of B*a*P occurred during the experiment. Preliminary tests showed that the recovery of B*a*P was greater than 90% after a 10-d desorption period (Table 1).

The water concentration measured by liquid scintillation counting includes B*a*P in the freely dissolved phase and B*a*P bound by the dissolved organic carbon (DOC) in the water. The DOC content in the water was determined using a Shimadzu TOC-5050A total organic carbon analyzer (Shimadzu, Columbia, MD, USA) in our previous experiment on phenanthrene-amended sediment. The average DOC content was 2.26 ± 0.40 mg/L (*n* = 9). Although this was shown to have a negligible impact on phenanthrene (X. Lu, unpublished data), a significant influence on dissolved-phase B*a*P is expected. With the measured DOC content, the measured water concen-

Table 1. Mass balance for benzo[*a*]pyrene in the desorption vials

Total mass[a] (dpm)	Measured total mass[b] (dpm)	Recovery (%)
343,857.0	316,692.3	92.1
344,961.3	325,298.5	94.3
345,237.3	328,665.9	95.2

[a] The total mass introduced into the system was calculated based on the initial activity of the sediment and the mass of the sediment introduced; dpm = disintegrations per minute.

[b] The measured total mass was calculated based on the measured activity in water and the remaining sediment after 10-d desorption.

tration of B*a*P was corrected assuming that the partition coefficient of DOC is approximately the same as that for the sediment organic matter [18]. Then, the measured water concentration follows as

$$C_{wm} = C_w + C_w f_{DOC} K_{OC} \qquad (1)$$

where C_{wm} and C_w are the measured and free or true water concentration (mg/L), respectively, and f_{DOC} and K_{OC} are the dissolved organic carbon content in water and organic carbon–normalized sediment–water partition coefficient of B*a*P, respectively.

The free or true water concentration can be written as

$$C_w = \frac{C_{wm}}{(1 + f_{DOC} K_{OC})} \qquad (2)$$

If the DOC content was 2.26 mg/L and the K_{oc} of B*a*P was taken as $10^{5.80}$ [10], then the correction factor (denominator in Eqn. 2) was approximately 2.5, which means that the measured water concentration of B*a*P was 2.5-fold the truly dissolved water concentration. The partition coefficient of B*a*P (K_p) was calculated using the above, corrected water concentration (C_w), and the remaining sediment concentration after 10-d desorption was calculated by mass balance.

Measurement of uptake kinetics

Measurement of the uptake kinetics of B*a*P was conducted in 50-ml glass tubes. In each tube, 15 worms of similar size were exposed to approximately 50 g of wet sediment (moisture content, ~40%). Time to apparent (or 95%) steady state (t_s) of the uptake of B*a*P was estimated based on the elimination rate of B*a*P (k_e) using the formula derived from the first-order kinetics model [19]: $t_s = \ln[1/(1.00 - 0.95)]/k_e$. Harkey et al. [20] has reported that the elimination rate of B*a*P in *Lumbriculus variegatus* was 0.0032 h^{-1}. Using these data, the estimated time to apparent steady state for the uptake of B*a*P was approximately 39 d. Therefore, the accumulation in the two sediments was repeatedly measured on days 5, 10, 16, 26, and 38 with three replicates for each sediment and exposure combination. Steady state was assumed to be reached if no statistically significant difference of the biota–sediment accumulation factors (BSAFs) was observed in the last two exposures. Uptake in short exposures was also measured (but only with the low-concentration sediment), and the uptake was measured after 2, 6, 12, 24, and 48 h. At each sampling time, three tubes from each sediment were sacrificed, the worms were sieved from the sediment, and survivors were enumerated and allowed to purge their digestive system for 6 h in clean artificial pond water (0.5 mM NaCl, 0.2 mM NaHCO₃, 0.05 mM KCl, and 0.4 mM C$_a$Cl₂). The [³H]B*a*P accumulated in the worms' tissue

was counted immediately in two groups with four or five worms in each group, and the other six worms in each tube were frozen for lipid analysis using the procedure described previously [12]. No fresh sediment was added to the tubes except for the longest exposure period (38 d), in which 50 g of sediment did not provide sufficient food for the worms during the whole period of exposure [19]. When adding sediment, the tubes were gently tapped on a three-layer paper tower to disturb the worms to burrow down to the bottom of the tube. Then, the rings and cheese-cloth were removed from the tubes, and the top layer of sediment was carefully taken out by a spatula, new sediment with the same B*a*P concentration added, and the side of the tubes cleaned by a soaked paper tower. When everything was ready, rings and cheese-cloth were replaced, the artificial pond water refilled, and the tubes were put back into the incubator.

Measurement of AE

Assimilation efficiency was measured using the pulse-chase feeding technique described by Selck et al. [21] and based on direct measurement of the [³H]B*a*P ingested and remaining in tissue after complete egestion. Twenty-four worms were first exposed to 15 ml of [³H]B*a*P-labeled sediment for 40 min (≤1 gut-passage time). Then, worms were gently but quickly taken out of the radiolabeled sediment, flushed with water to remove sediment-associated isotope adsorbed on the surface of the body, and divided into two groups: From each replicate, nine worms were placed in an ingestion group, and 15 worms were placed in a depuration group. Worms in the ingestion group were immediately subjected to liquid scintillation counting in groups of three worms. The average of the total count normalized by weight of the three subsamples was taken as the ingested sediment concentration (F_i). The worms in the depuration group were moved to the unlabeled sediment to purge the ingested materials. Five worms were counted together to ensure that high counts could be obtained after depuration, and the average of the count was taken as the B*a*P concentration assimilated into the worms' tissue (F_t). Then, AE was calculated as F_t/F_i.

Worms in the depuration group were transferred to sediment without B*a*P and allowed to depurate for 4, 8, 24, and 48 h to determine when gut clearance occurred. At each sampling time, 15 worms were removed, and tissue concentration was measured by liquid scintillation counting. The fraction of mass remaining after depuration was calculated at each period, and complete egestion was estimated from the profile of this fraction. Assimilation efficiency was calculated as the fraction of the remaining body burden on complete egestion, which was estimated to be 4 h.

Elimination and biotransformation

In the elimination experiment, 60 *I. templetoni* were exposed to the contaminated sediment for 7 d. After placement in artificial pond water for 9 h to allow gut clearance, individuals were transferred to clean sediment and analyzed for B*a*P body burden after 0, 13, 37, 62, 90, and 115 h. Data were fitted by a first-order decay model, and the elimination rate constant was determined from the model. Elimination determines the total loss of B*a*P from the worms' tissue, including both active and passive processes.

A biotransformation experiment was conducted to define the metabolism of B*a*P by the worms or active loss of B*a*P. Twenty-four worms were exposed to contaminated sediment;

sampled on days 6, 17, 24, and 38; and divided into two subsamples. Tissue extraction followed the procedure used by Millward et al. [22], which was adapted from that of Landrum [23] by Lotufo [24]. The extracts were then applied to a thin-layer chromatographic plate (Whatman LK6DF, silica gel 60 A; Whatman, Maidstone, Kent, UK). The plate was developed in a glass chamber with 50 ml of hexane:benzene (9:1 [v/v]). Benzo[*a*]pyrene isolates were identified under ultraviolet light and collected by removing the silica gel with a scalpel. The remaining silica gel associated with B*a*P metabolites were separated by 3 cm and collected in different scintillation vials to avoid the lumex effect when counting in 6 ml of scintillation cocktail, and the counts from each vial were combined as the total count of B*a*P metabolites. The fraction of B*a*P metabolites in tissues was estimated using the ratio of disintegrations per minute (dpm) not associated with the parent B*a*P to total disintegrations per minute.

Analyses

In the present study, all measurements were based on the radioactivity of the samples because of the simplicity of preparing samples and the high sensitivity of analysis.

For each sediment sample, 5 to 15 mg of dry ground sediment were used and put into a 25-ml glass scintillation vial. Before adding 10 ml of scintillation cocktail (Biosafe II; Research Products International, Mount Prospect, IL, USA), 1 ml of distilled water was added to each sample, which was then shaken well by hand.

For tissue concentration measurement, worms with cleared guts were transferred to 8-ml scintillation vials, dissolved by 200 μl of tissue solubilizer (TS-2, 0.5 N solution; Research Products International), and placed on a warm plate overnight. One-hundred microliters of 1.2 N HCl were added to neutralize the tissue solubilizer before adding 6.0 ml of scintillation cocktail.

The activity of sediment and tissue samples were then determined using a Beckman LS 6000IC liquid scintillation counter (Beckman-Counter, Fullerton, CA, USA) and measured as disintegrations per minute. The data were corrected for quenching using the external standards ratio method after subtracting the background.

Data analysis

Accumulation data were analyzed using both an equilibrium partitioning bioaccumulation model defined by the BSAF and a first-order kinetic model [25]. The ratio of lipid-normalized tissue concentration to organic carbon–normalized sediment concentration was calculated at each exposure period, and the ratio at steady state defined the observed BSAF. The normalized accumulations were calculated for each replicate based on the measured average tissue concentration of B*a*P and the average lipid content of the worms for each replicate test tube. Two-way analysis of variance was used to analyze the influence of sediment treatment and exposure period on the normalized accumulation. An expected, BSAF can be estimated on the basis of the expected pore-water concentration and the partition coefficient between lipid and water, as shown by Lu et al. [12]

When sediment is the source compartment, a kinetic model of uptake can be written as

$$\frac{dC_t}{dt} = k_s C_s - k_e C_t \qquad (3)$$

Table 2. Sediment characteristics of the inoculated sediment and desorbed sediments[a]

Sediment[b]	Benzo[*a*]pyrene concn. (μg/g)	Activity (dpm/mg)[c]	f_{OC} (%)[d]	Log K_{OC}[e]
S_0	60.53 (±0.96)	1,148.42 (±19.14)	1.37	6.12 (±0.03)
S_1	25.85 (±1.46)	414.30 (±9.53)	1.25	6.21 (±0.06)
S_2	16.24 (±1.92)	311.79 (±11.73)	1.20	6.25 (±0.04)
S_3	10.90 (±0.41)	232.13 (±35.17)	1.20	6.26 (±0.06)

[a] The numbers in parentheses represent one standard deviation from the mean. The calculations for sediment concentration and K_{oc} were based on four and three replicates, respectively. There were only two replicates in the organic carbon measurement.
[b] S_0, S_1, S_2, and S_3 denote originally inoculated sediment and sediments desorbed once, twice, and three times, respectively.
[c] dpm = disintegrations per minute.
[d] f_{OC} = fraction of organic carbon content of the sediment.
[e] K_{OC} = organic carbon normalized sediment–water partition coefficient.

where C_t is the B*a*P concentration accumulated in the worms' tissue (dpm/mg dry tissue), C_s is the sediment concentration (dpm/mg dry sediment), k_s is the uptake rate coefficient from the sediment (g sediment·g worm^{-1}·h^{-1}), and k_e is the elimination rate constant (h^{-1}).

Assuming that sediment concentration is constant during exposure and integrating Equation 3,

$$C_t = \frac{k_s \cdot C_s}{k_e}[1 - \exp(-k_e t)] \qquad (4)$$

Fitting the uptake kinetics data with Equation 4 using a nonlinear regression technique provided by SigmaPlot (SPSS Science, Chicago, IL, USA) gave the estimated value of the uptake rate coefficient (k_s) and the elimination rate constant (k_e).

Biota–sediment accumulation factor can be calculated (BSAF$_{calc}$) from the kinetics model as time approaches infinity:

$$\text{BSAF}_{calc} = \frac{k_s}{k_e} \frac{f_{OC}}{f_{lipid}} \qquad (5)$$

where f_{OC} and f_{lipid} are the fraction of total organic carbon content of the sediment and the fraction of lipid content of the worms' tissue, respectively. Both are presented on a dry weight basis in the present study.

Elimination of B*a*P from worms was described by a first-order decay model:

$$C_t = C_{t=0} \cdot e^{(-k_e t)} \qquad (6)$$

Half-life ($t_{1/2}$) and the time to eliminate 99% of body burden (t_{99}) can be calculated as

$$t_{1/2} = \frac{\ln 2}{k_e} \qquad t_{99} = \frac{\ln(100)}{k_e} \qquad (7)$$

RESULTS

*Sediment characteristics and desorption isotherm of B*a*P*

Sediment characteristics, including B*a*P concentration and activity, organic carbon content (f_{oc}), and organic carbon–normalized sediment–water partition coefficients (K_{oc}) for the originally inoculated sediment (S_0) and three desorbed sediments (S_1, S_2, and S_3) are listed in Table 2. Organic carbon content (f_{oc}) of the sediment was reduced approximately 9% during the first-batch desorption but was essentially constant in the subsequent desorptions. The reduction of organic carbon
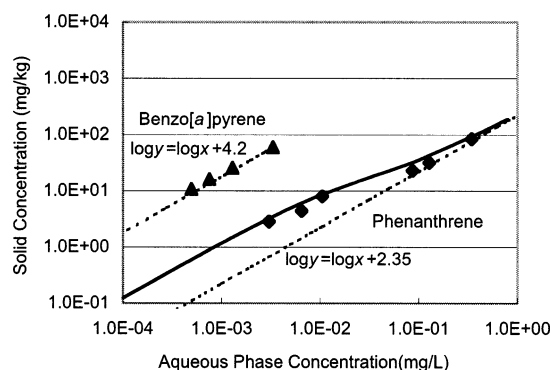
Fig. 1. Desorption isotherm of benzo[*a*]pyrene and phenanthrene. The filled diamonds and triangles are experimental data. The solid line is the desorption of phenanthrene predicted by the biphasic model of Kan et al. [8]. The two dashed lines are the desorption isotherms predicted by the linear partitioning model. E = exponent to the base 10.

Fig. 3. Lipid-normalized tissue concentration over organic carbon–normalized sediment concentration at each exposure period. Biota–sediment accumulation factor was approximately 1.3 for both sediments at steady state. Error bars represent one standard deviation from the mean. S_1 = sediment desorbed for one batch; S_3 = sediment desorbed for three batches.

content probably resulted from the removal of fine particles after centrifugation. Desorption isotherm of B*a*P was determined based on the measured solid concentration and the pore-water concentration of the four sediments (Fig. 1). Compared to the desorption of phenanthrene [12], no apparent desorption resistance was observed, because the equilibrium desorption isotherm of B*a*P was identical to that estimated by reversible desorption, which was calculated by a linear partitioning model [26] using the partition coefficient of the originally inoculated sediment.

Bioaccumulation

Tissue concentration of B*a*P was higher than the sediment concentration even after short exposures (24 h), and body burden increased with increasing sediment concentration. Uptake of B*a*P was almost linear during the short exposure (<2 d), and the accumulation of B*a*P attained an apparent steady state after approximately one month for S_1 and S_3 sediments (Fig. 2). Although BSAF was defined at steady state, the same normalization method was used at each exposure period to observe the change of the normalized accumulation before the BSAF was obtained (Fig. 3). Results of two-way analysis of variance showed significant interaction between time and sediment treatment, indicating that no parallel trend of sediment effect on the normalized accumulation over the exposure period was
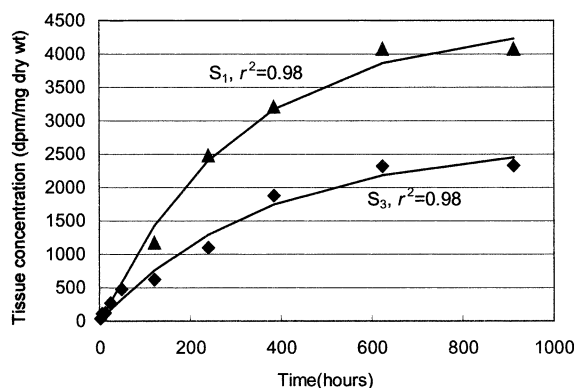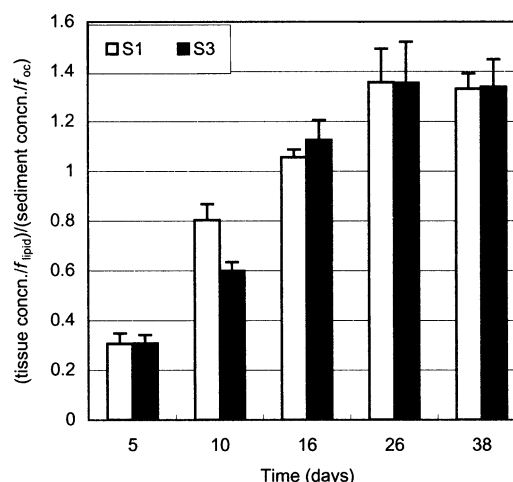
observed ($p = 0.0013$, $df = 4$). Pairwise comparisons were made using a posteriori Tukey test, and sediment treatment effect was analyzed at each time slice. The normalized accumulation increased with time until the last two exposures, suggesting that steady state was reached in approximately one month. No significant sediment–treatment effect was observed on the normalized accumulation of B*a*P except at 10-d exposure ($p < 0.0001$). At steady state, worms in S_1 and S_3 sediments attained a BSAF of 1.33 ± 0.06 ($n = 3$) and 1.34 ± 0.11 ($n = 3$), respectively. The uptake kinetics of B*a*P was fit by a first-order kinetic model (Eqn. 4). The model fit very well for both sediments, with high correlation coefficients ($r^2 = 0.98$ for both) (Fig. 2). The uptake rate coefficients and elimination rate constants from the model fit were 0.035 and 0.032 g sediment·g worm^{-1}·h^{-1} and 0.0032 and 0.0028 h^{-1}, respectively. The BSAFs calculated from the estimated uptake rate coefficient and elimination rate constant (Eqn. 5) were 1.48 and 1.50 for sediments S_1 and S_3, respectively, which were very close to the BSAF measured at steady state (Table 3). The high correlation of fit by the kinetic model and the agreement between the predicted BSAF with the experimentally measured BSAF suggested that the first-order kinetic model is appropriate to describe the accumulation of sediment-associated B*a*P by *I. templetoni*.

Assimilation efficiency

In the depuration group, the fraction of B*a*P remaining in the worms' tissue decreased from 100% with all ingested sed-

Fig. 2. Uptake kinetics of benzo[*a*]pyrene in the sediments that have been desorbed for one and three batches (S_1 S_3, respectively). The solid lines are the results fitted by Equation 4. dpm = disintegrations per minute.

Table 3. Comparison of calculated biota–sediment accumulation factor (BSAF) from the kinetic data with measured BSAF

Sediment[a]	Model fitted k_s (g · g^{-1} · h^{-1}) and k_e (h^{-1})	BSAF	
		Calculated	Measured[b]
S_1	0.035 and 0.0032	1.48	1.33 (±0.06)
S_3	0.032 and 0.0028	1.50	1.34 (±0.11)

[a] S_1 = single batch desorption; S_3 = three sequential batch desorptions.
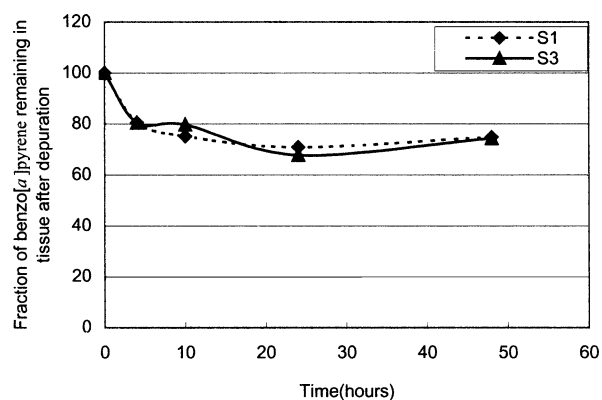[b] The numbers following in parentheses represent one ± SD from the mean.

Fig. 4. Fraction of [³H]benzo[*a*]pyrene remaining in worms at different depuration periods. Assimilation efficiency as defined by the fraction of body burden remaining at the conclusion of sediment egestion is approximately 80% for benzo[*a*]pyrene by *I. templetoni*. S_1 = sediment desorbed for one batch; S_3 = sediment desorbed for three batches.

iments still in the worms' digestive system at the beginning of the depuration period to 80% after 4 h of depuration in unlabeled sediment, and this value became almost stable after that time for both sediments (Fig. 4). The rapid loss during the first 4 h probably resulted from digestive clearance (purging), and the subsequent slow changes in concentration shown in Figure 4 likely were caused by elimination from the worms' tissue. Assimilation efficiency, as defined by the fraction of body burden remaining at the conclusion of sediment egestion, was approximately 80% for B*a*P, indicating that 80% of ingested sediment–associated B*a*P would be taken up by the organism during a single gut-passage.

Elimination and biotransformation

Elimination of B*a*P from worms was slow but followed a first-order pattern during the 115-h elimination period (Fig. 5). Data were fit by Equation 6 with correlation coefficients of 0.88 and 0.96, respectively. The derived elimination rate constants (k_e) were 0.0066 and 0.0064 h⁻¹ for S_1 and S_3 sediments, respectively, indicating that elimination of B*a*P was independent of tissue concentration in this case. The measured elimination rate constant was approximately twofold that derived from the uptake kinetics data (Eqn. 6), because the elimination rate constant measured in this experiment reflected loss
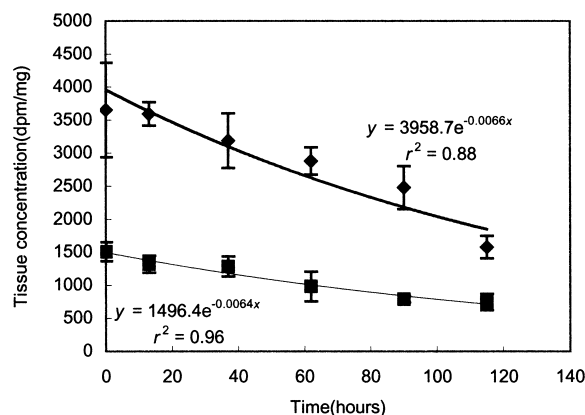

Fig. 5. Time course of body burden of benzo[*a*]pyrene in clean sediment after 7-d exposure to contaminated sediment and 9-h gut purging in artificial pond water. Solid lines are the results of fit by the first-order model (Eqn. 6), and error bars represent one standard deviation from the mean. dpm = disintegrations per minute.

of B*a*P from the body in the absence of B*a*P, which was actually the depuration rate. However, the elimination rate derived from fitting of the uptake kinetics reflected the loss in the presence of B*a*P. The half-life and time to 99% elimination of B*a*P, based on the model fitted k_e, were approximately 10 and 64 d, respectively. The result of biotransformation was consistent with the slow elimination of B*a*P. Less than 6% of the parent B*a*P was metabolized by *I. templetoni* during the 38-d exposure, and no strong trend over time was observed, indicating that B*a*P resists metabolism by *I. templetoni*.

DISCUSSION

Our empirically derived measure of the desorption isotherm of B*a*P demonstrated no significant resistance in the desorption of sediment-associated B*a*P. This result is consistent with the prediction of the biphasic model of Kan et al. [8]. The model states that equilibrium desorption resistance, as indicated by increases in apparent partition coefficient, becomes less pronounced when the hydrophobicity of a compound increases, especially when the K_{OC} of an organic contaminant is greater than the predicted K_{OC}^{res} ($10^{5.53\pm0.48}$ ml/g), in which case almost no resistance occurs in the desorption of the contaminant.

Although B*a*P exhibited high partitioning to the solid phase, sediment-associated B*a*P was available to *I. templetoni*. Accumulation of B*a*P reached a very high tissue concentration at steady state and a BSAF of approximately 1.3 for both sediments. The BSAF of B*a*P measured in the present study was close to that demonstrated by another deposit-feeding annelid, the polychaete *Leitoscoloplos fragilis*, at a concentration less than 1 μg B*a*P/g [27]. However, the BSAF of B*a*P in *I. templetoni* was much higher than the values for the polychaetes *Nereis diversicolor* and *Scolecolepides viridis*, potentially because of PAH metabolism [27]. This implies that if metabolism of the contaminant is negligible and no toxicity is observed, BSAF would be independent of species and sediment concentration.

In previous research, we developed a model that predicts bioaccumulation (BSAF) based on a contaminant's effective partition coefficient from a variety of laboratory results with phenanthrene, pyrene, and literature data in which sorbents were used to control pore-water concentration [12]. Oligochaetes, on which the model is based, likely do not readily metabolize PAHs [22; present study]. These data suggested that bioaccumulation is well predicted by partitioning into the sediment pore-water phase and that the effective partition coefficient could be used to predict the steady-state bioaccumulation. In the present study, the two desorbed B*a*P sediments, S_1 and S_3, exhibited equivalent sediment–water partition coefficients and almost-identical normalized accumulation. This finding is consistent with the paradigm that the effective pore-water concentration controls the bioaccumulation of hydrophobic contaminants by deposit-feeding organisms for a given compound, even in the case of B*a*P, in which the primary route of exposure is ingestion of sediment [28–30] (Fig. 6 expands the previous relationship by adding B*a*P data using the predictions based on pore-water concentrations as shown by Lu et al. [12]). This result is also consistent with the recent results of Kraaij et al. [31], who identified pore-water concentrations as a good predictor of accumulation in a variety of compounds. In this case, the two-stage uptake includes partitioning to the digestive fluid and uptake from the gut to tissues. Surfactants in the gut fluid may enhance release of the contaminant and, potentially, increase measures of single
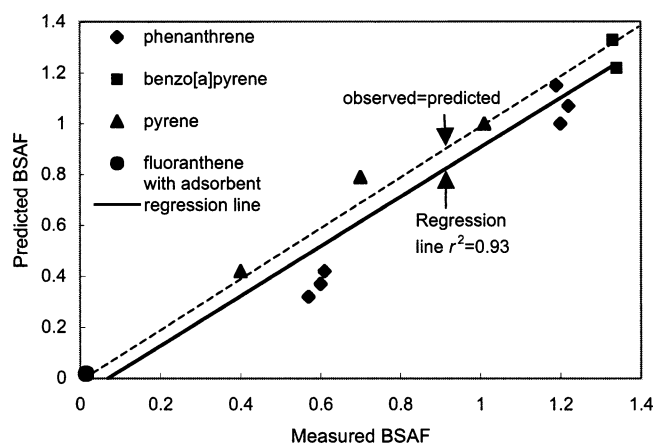
Fig. 6. Biota–sediment accumulation factor (BSAF) predicted from the effective partition coefficient versus measured BSAF for benzo[*a*]pyrene, phenanthrene, pyrene, and fluoranthene. Information for the other three compounds was described previously [12].

gut-passage absorption efficiency and rate of uptake. Digestive fluid, as an intermediate phase, however, cannot influence the ultimate partitioning between the two phases: sediment and tissue. Thus, the ultimate or steady-state bioaccumulation of B*a*P in the present study and that of phenanthrene in the previous experiments [12] were observed to be independent of the measured AE and controlled only by partitioning in sediment, water, and lipid systems. As with any three-phase system in physical equilibrium, partitioning between any two phases defines the partitioning to the third phase. Increases in uptake without corresponding increases in body burden may result from exchange through the body wall with surrounding pore water (i.e., an increase in elimination at high rates or extent of uptake to maintain equilibrium between the phases). This conclusion has been developed only with soft-bodied, deposit-feeding benthic oligochaetes that are not expected to metabolize PAHs; thus, contaminant uptake is largely associated with partitioning. The applicability of the model to other benthic organisms and other contaminants needs to be explored.

The first-order kinetics model, which used sediment as a source compartment, fit the uptake kinetics data of B*a*P very well ($r^2 = 0.98$), and the BSAFs of the sediments S_1 and S_3 calculated from this kinetics model were very close to the experimentally measured values. However, this kinetics model cannot distinguish the uptake from sediment particles and the uptake from the pore water. The uptake rate coefficient (k_s) integrates uptake from all sediment phases and is a measure of the overall bioavailability of the contaminant in particular sediment [32]. A bioenergetic-based toxicokinetic model [25], assuming that uptake from each source is independent from each other and proportional to contaminant concentration, its flux, and uptake efficiency, can theoretically estimate the uptake from each different route. Assimilation efficiency was used to define the efficiency of the uptake from ingested sediment. Difficulty in the measurements of the AE and the large variability in the results limit the accuracy of the model. Assimilation efficiency will vary with elimination rate and the organism's ability to metabolize the contaminants; thus, it will vary greatly from species to species. Different methods may also contribute to differences in values, as shown by Kukkonen and Landrum [33]. Additionally, AE will vary if measurements are made over longer time periods in at least some animals

because of elimination and inducible enzyme pathways. Our data suggested that the elimination rate of B*a*P was slow and that no significant metabolic degradation of B*a*P occurred. This may contribute to the high AE found for B*a*P in the present study.

CONCLUSION

Sediment-associated B*a*P was available to the deposit-feeding, freshwater oligochaete *I. templetoni*, although extreme toxicity was observed at the concentration of 60μg B*a*P/g sediment. No obvious resistance, as measured by desorption isotherm, was observed for B*a*P, which resulted in almost-identical normalized accumulation at the high and low concentrations of the desorbed sediments. This is consistent with the paradigm that effective pore-water concentration controls the uptake of hydrophobic organic contaminants in deposit-feeding organisms. This was previously observed for phenanthrene, which is expected to be absorbed primarily from pore water [28], but has now been shown for the uptake of B*a*P, which is expected to be absorbed primarily from ingested sediment [28–30]. The uptake of B*a*P attained an apparent steady state after almost one month and resulted in a BSAF of approximately 1.3, which was a combined result of high AE (80%), low elimination rate ($k_e \sim 0.0032$ h^{-1}), and negligible biotransformation of B*a*P in the *I. templetoni* worms. The first-order kinetics model fit the uptake data well but could not distinguish uptake from sediment particles and pore water.

Assimilation efficiency defines the rate of uptake, whereas steady-state accumulation under the conditions of the present study is defined by simple partitioning. The pore-water concentration in the sediment can then be used to define the steady-state accumulation despite ingestion being the primary route of uptake. Desorption resistance reduces bioavailability and bioaccumulation of these partitioning contaminants by reducing pore-water concentration and producing a corresponding reduction in steady-state accumulation. This conclusion would be complicated by metabolic processes that might occur with other compounds or in other organisms that would introduce fate processes other than simple partitioning.

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